


TRITC-transferrin internalization assay

FL Feng Liu

Updated date: Jun 24, 2021

 An abbreviated version of this protocol was published in PLoS Biology in Apr 2020

Rab5c-mediated endocytic trafficking regulates hematopoietic stem and progenitor cell development via Notch and AKT signaling

DOI: 10.1371/journal.pbio.3000696

Detailed protocol

1. HeLa cells were plated on optical borosilicate glass coverslips (Thermo) and cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo), supplemented with 10% fetal bovine serum (Thermo), 100 U/mL penicillin and 100 mg/mL streptomycin (Thermo) at 37°C, 5% CO₂.
2. When the cells grown to about 50% confluent, the indicated plasmids were transfected into cells using lipofectamine 3000 (Invitrogen), according to the manufacturer's instructions.
3. Cells transfected with indicated plasmids were serum-starved for 6 h. Then the culture dish was kept on ice for 10 min.
4. Removed the serum-free DMEM and incubated cells in PBS containing 10 µg/mL of TRITC-conjugated transferrin (Rockland), 1% BSA (Sigma-Aldrich) and 0.3% Hoechst 33342 (Invitrogen) for 10 min at 37°C, then removed the uptake medium and quickly washed 3 times with ice-cold PBS.
5. The cells on the glass coverslips were observed with a Nikon A1 confocal microscopy (Nikon). TRITC was excited at 561 nm and Hoechst 33342 was excited at 405 nm line of laser. Red and blue emissions were collected simultaneously. Pictures were taken by an Nikon A1 confocal camera system.
6. The fluorescence intensities were analyzed using ImageJ. The Student t test was used for statistical comparisons. Plotted mean was calculated by GraphPad software.

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Liu, F. (2021). TRITC-transferrin internalization assay. Bio-protocol Preprint. bio-protocol.org/prep1202.
2. Heng, J., Lv, P., Zhang, Y., Cheng, X., Wang, L., Ma, D., Liu, F., Jiang, D., Jiang, D., Jiang, D. and Jiang, D. (2020). Rab5c-mediated endocytic trafficking regulates hematopoietic stem and progenitor cell development via Notch and AKT signaling. PLoS Biology 18(4). DOI: [10.1371/journal.pbio.3000696](https://doi.org/10.1371/journal.pbio.3000696)

Copyright: Content may be subjected to copyright.